A PROBE INTO THE MECHANISM OF PREFERENTIAL CONCENTRATION OF DL-ALANOSINE (NSC-143647) IN THE BRAIN OF THE SPINY DOGFISH

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In earlier studies (MDIBL Bull. 16:47, 1976) it has been shown that for drugs with positive neoplastic activity there exists a good correlation between 24 hour dogfish brain tissue/plasma ratios of an anti-cancer drug and drug effectiveness against a mouse brain tumor system. Of the effective drugs tested, DL-alanosine (NSC-143,647) far surpassed all others in its ability to be taken up and concentrated in the brain and CSF of the dogfish. The brain to plasma ratio at 24 hours was 43.9 as measured by the radio-activity retained in the respective tissues. This ratio is about 40 times greater than that of CCNU, the only agent with established brain tumorilytic activity in humans (J. Neurosurg. 37:306, 1973). Since this drug is an amino acid (aspartic acid) analog, it was of primary importance to further characterize the nature and form of the radioactivity retained in the brain in order to know the mechanism of retention.

Dogfish were given i.v. DL-alanosine along with DL-1-[^{14}C]alanosine (10 mg/Kg) and urine and brain tissue were collected at 4 and 24 hours. Four-hour brain and urine samples were used for the analytical studies reported here. High voltage paper electrophoresis (HVPE) and ascending paper chromatography were done to confirm some of the column chromatography results. The automated column chromatography using the amino acid analyzer was carried out on a Hamilton HA x 4, anion exchange column (8 x 150 mm), the elution being carried out with a series of five citrate buffers of increasing salt concentration.

A 5% perchlorate extract of the shark brain from control as well as alanosine-treated animals was prepared and was neutralized. Drug-treated shark urine or shark brain PCA extract were fractionated on the anion exchange column. Panel A of the figure is a standard chromatogram indicating the position of elution of the parent compound and mouse urinary metabolite. (Mouse urinary metabolite was used as a standard, since it is the major metabolite from the monkey, dog, rat and mouse.) DL-Alanosine elutes at 12 min and the metabolite at 82 min. Panel B of the figure shows the elution profile of urine from DL-(1-^{14}C)alanosine-treated sharks.

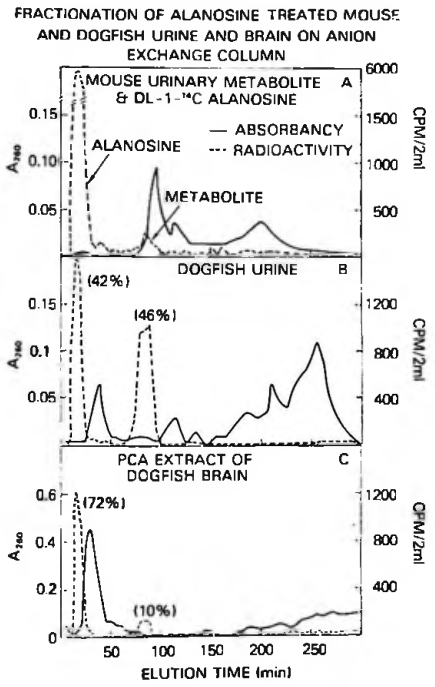

Figure 1. Chromatography of dogfish urine, neutralized PCA extract of alanosine treated dogfish brain, or partially purified mouse urinary metabolite and ^{14}C-alanosine was separately carried out on the anion exchange column as described earlier. Five minute fractions were collected and the radioactivity was determined by counting 2 ml of each fraction using 10 ml of quench resistant scintillation cocktail (#3a70B from Research Products International).

Forty-two percent of the radioactivity eluted as parent compound and 46% eluted as metabolite. Panel C of the figure shows the elution profile of PCA brain extract of DL-(1-^{14}C)alanosine-treated shark, 72% of

radioactivity eluted as parent compound and 10.6% eluted as the metabolite. Shark urinary metabolite when analyzed by HVPE or by paper chromatography showed similarity to the urinary metabolites of dog, monkey, rat and mouse. It was ninhydrin negative, unlike alanosine and had retained the UV absorption characteristics of the parent compound. Since the urinary metabolite and brain metabolite behaved similarly on anion exchange column, we presume that the brain metabolite shares these characteristics as well.

The observation that the majority of the radioactivity present in the brain is in the form of the parent compound diminished the possibility that a metabolite less soluble than the urinary metabolite or the parent compound was responsible for accumulation of radioactivity in the brain. Hence, a few simple experiments were conducted to find out if the bulk of the radioactivity (present as the parent compound) is retained by any preferential binding of the same to the macromolecules. Twelve thousand g supernatant of the brain carried 67% of the total radioactivity and the rest was with the pellet. Six percent of the radioactivity associated with the pellet could further be released if the homogenate was made 5% with respect to PCA prior to centrifugation. An additional 15% of this associated radioactivity could be removed after repeated washing of the pellet with methanol. These figures indicate that only a low percentage of the parent compound and/or metabolite is actually associated with and presumably incorporated into proteins or nucleic acids. Exhaustive dialysis of the brain homogenate indicated that about 70% could be dialyzed out. The amount of non-dialyzable radioactivity is once again the same as that associated with the pellet. It was therefore necessary to find out whether the residual radioactivity associated with the pellet was due to any preferential binding of the drug to macromolecules present in the brain. This was achieved by doing an equilibrium dialysis experiment. Control brain extract was dialyzed against buffer containing DL-1-[^{14}D]alanosine. Analysis of the contents of the bag and the dialysate showed that there was no preferential binding of the drug to the macromolecules of the shark brain. Non-dialyzable radioactivity is therefore associated with macromolecules by interactions which are not ionic and different from other non-covalent bindings.

Though more lipophilic forms have a better chance of being retained in the brain, small molecules similar to amino acids have good chances of entering the brain. In fact, the behavior of L-alanosine in neonatal mouse brain causing hypothalamic lesions similar to the ones caused by high doses of L-aspartic and L-glutamic acids (Proc. Amer. Assoc. Cancer Res. 18:219, 1977) indicates how similar this drug is to these amino acids. Though the present analysis does not say the final word on the mechanism of retention of L-alanosine, however, it has opened up possibilities for water soluble drugs for effective treatment versus brain tumor. Nonetheless, on the strength of the efficacy of this drug against mouse brain tumor and these results reported here, it has been recommended by the Drug Development Program of NCI for early clinical trials against human brain tumors.

EFFECTS OF WATER SOLUBLE FRACTIONS FROM CRUDE OILS ON DEVELOPMENT OF SAND DOLLAR EMBRYOS

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Until the supplies of petroleum are exhausted, marine life will be threatened periodically by accidental spillage of crude oil. Therefore it is pertinent that effects of water-soluble components from these oils be observed in a variety of marine organisms. The developing echinoderm embryo is a potentially useful one for determining effects of pollutants, since the eggs and sperm can be readily obtained and the embryo can be reared under controlled conditions.

For the studies reported here gametes were obtained from the sand dollar, *Echinarachnius parma*. after injection of about 1 ml of 0.5 M KCl into the mouth area of the animal. Eggs from a single female were washed in a 250 ml beaker and then fertilized by adding 10 drops of a 1% suspension of sperm. Incubation embryo suspensions was carried out in filtered sea water at 16°. Data from an experiment were accepted on